## REMARKS

Certain paragraphs in the specification and claims 5, 23, 24 and 25 have been amended to insert the sequence identifier numbers and to reflect that Figure 8 is in two parts.

No new matter has been introduced by the amendments to the specification.

## CONCLUSION

Applicants respectfully request that the above-made remarks be made of record in the file history of the present application. The claims fully meet all statutory requirements for patentability.

Applicant respectfully requests that the Examiner call the undersigned at (212) 790-9090 if any questions or issues remain.

Respectfully submitted,

Date March 7, 2003

Geraldine F. Baldwin 31,232
Geraldine F. Baldwin (Reg. No.)

By: William Thomann 40,203
William Thomann (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

# EXHIBIT A

Serial No.: 09/884,098
Filed: June 20, 2001
Attorney Docket No.: 9426-022

**MARKED-UP VERSION OF THE SPECIFICATION**
**DOUBLE UNDERLINED TEXT IS ADDED**
**AND [BRACKETED TEXT] IS DELETED**

On page 22, line 7 replace the paragraph beginning with "Figure 8" with the following:

**Figures 8A-8B [Figure 8]:** BSP Promoter Sequence from -2184 to +237 (SEQ ID NO:1).

On page 30, lines 16-23, replace the paragraph beginning with "The present invention" with the following:

The present invention encompasses polynucleotide sequences comprising the 5' regulatory region, and transcriptionally active fragments thereof, of the BSP gene. In particular, the present invention provides polynucleotides comprising 907 bp, 1107 bp, 1418 bp, 1459 bp and 2253 bp sequences that are located within a BSP gene. Specifically, the polynucleotides comprise -838 bp through +69 bp, -1038 bp through +69 bp, -1349 bp through +69 bp, -1390 bp through +69 bp and -2184 bp through +69 bp, respectively, of the BSP sequence shown in [Figure 8] Figures 8A-8B (SEQ ID NO:1). In various embodiments, the polynucleotide may be 5000, 4000, 3000, 2000, 1000 and preferably approximately 500 bp in length.

On page 31, lines 20-24, replace the paragraph beginning with "The nucleotide sequences" with the following:

The nucleotide sequences of the invention also include nucleotide sequences that have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more nucleotide sequence identity to the nucleotide sequence depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1), and/or transcriptionally active fragments thereof, which are capable of driving expression specifically within tumor and tissue cells with calcification potential.

On page 33, lines 1-11, replace the paragraph beginning with "Also encompassed within" with the following:

Also encompassed within the scope of the invention are various transcriptionally active fragments of this regulatory region. A "transcriptionally active" or "transcriptionally functional" fragment of the sequence depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1) according to the present invention refers to a polynucleotide comprising a fragment of said polynucleotide which is functional as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide in a recombinant cell host. For the purpose of the invention, a nucleic acid or polynucleotide is "transcriptionally active" as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide if said regulatory polynucleotide contains nucleotide sequences which contain transcriptional information, and such sequences are operably associated to nucleotide sequences which encode the desired polypeptide or the desired polynucleotide.

On page 33, line 31 to page 34, line 22, replace the paragraph beginning with "Also encompassed within" with the following:

Also encompassed within the scope of the invention are modifications of this nucleotide sequence without substantially affecting its transcriptional activities. Such modifications include additions, deletions and substitutions. In addition, any nucleotide sequence that selectively hybridizes to the complement of the sequence depicted in [Figure 8] Figures 8A-8B (SEQ IDNO:1) under stringent conditions, and is capable of activating the expression of a coding sequence specifically within tumor and tissue cells with calcification potential is encompassed by the invention. Exemplary moderately stringent hybridization conditions are as follows: prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/mℓ denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 µg/mℓ denatured salmon sperm DNA and 5-20 X $10^6$ cpm of $^{32}$P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Alternatively, exemplary conditions of high stringency are as follows: *e.g.*, hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1

mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3). Other conditions of high stringency which may be used are well known in the art. In general, for probes between 14 and 70 nucleotides in length the melting temperature (TM) is calculated using the formula: Tm(°C)=81.5+16.6(log[monovalent cations (molar)])+0.41 (% G+C)-(500/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature is calculated using the equation Tm(°C)=81.5+16.6(log[monovalent cations (molar)])+0.41(% G+C)-(0.61% formamide)-(500/N) where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below Tm (for DNA-DNA hybrids) or 10-15 degrees below Tm (for RNA-DNA hybrids).

On page 38, line 28 to page 39, line 13, replace the paragraph beginning with "Methods for assaying" with the following:

Methods for assaying promoter activity are well-known to those skilled in the art (see, *e.g.*, Sambrook *et al.*, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). An example of a typical method that can be used involves a recombinant vector carrying a reporter gene and genomic sequences from the BSP sequence depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1). Briefly, the expression of the reporter gene (for example, green fluorescent protein, luciferase, β-galactosidase or chloramphenicol acetyl transferase) is detected when placed under the control of a biologically active polynucleotide fragment. Genomic sequences located upstream of the first exon of the gene may be cloned into any suitable promoter reporter vector. For example, a number of commercially available vectors can be engineered to insert the BSP regulatory region of the invention for expression in mammalian host cells. Non-limiting examples of such vectors are pSEAP-Basic, pSEAP-Enhancer, pβgal-Basic, pβgal-Enhancer, or pEGFP-1 Promoter Reporter vectors (Clontech, Palo Alto, CA) or pGL2-basic or pGL3-basic promoterless luciferase reporter gene vector (Promega, Madison, WI). Each of these promoter reporter vectors include multiple cloning sites positioned upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline phosphatase, green fluorescent protein, luciferase or β-galactosidase. The regulatory sequences of the BSP gene are inserted into the cloning sites upstream of the reporter gene in both orientations and

introduced into an appropriate host cell. The level of reporter protein is assayed and compared to the level obtained with a vector lacking an insert in the cloning site. The presence of an elevated expression level in the vector containing the insert with respect the control vector indicates the presence of a promoter in the insert.

On page page 66, lines 14-29, replace the paragraph beginning with "Construction of the" with the following:

Construction of the recombinant Ad-BSP-TK virus was accomplished as shown in FIG. 1. All plasmids were constructed according to standard protocols. Briefly, pΔE1SP1, a shuttle vector which contains the 5' end part of the adenovirus genome with the El-region deleted, was digested witb Xho-1 (New England Biolabs, Beverly, Mass.) and treated with alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) according to the supplier's protocols. The 1418 bp BSP fragment was prepared using PCR. Specifically, a first primer set was used to amplify a 1467 bp insert. These primers were:

BSP1 - GTGGCACATATACACCATGG (SEQ ID NO:2)
BSP2 - AATCTTACCCTCTGGCAGTC (SEQ ID NO:3).

Internal primers to the 1467 bp fragment were then used to generate the 1418 bp BSP promoter. The internal primers were:

BSP3 - CCATGGAATACTATGCAGCC (SEQ ID NO:4)
BSP4 - TGGAGTGAGGAAGCAGGCTC (SEQ ID NO:5).

# EXHIBIT B

Serial No.: 09/884,098
Filed: June 20, 2001
Attorney Docket No.: 9426-022

**MARKED-UP VERSION OF THE CLAIMS**
**DOUBLE UNDERLINED TEXT IS ADDED**
**AND [BRACKETED TEXT] IS DELETED**

5 (once amended). The therapeutic agent of claim 1, wherein said BSP promoter comprises nucleotides -1349 to +69 depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1).

23 (once amended). The method of claim 18, wherein said BSP regulatory region sequence comprises nucleotides -1349 to +69 depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1).

24 (once amended). The method of claim 23, wherein said BSP regulatory region sequence comprises a nucleotide sequence which hybridizes under highly stringent conditions to the complement of nucleotides -1349 to +69 depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1).

25 (once amended). The method of claim 23, wherein said BSP regulatory region sequence comprises a nucleotide sequence which hybridizes under moderately stringent conditions to the complement of nucleotides -1349 to +69 depicted in [Figure 8] Figures 8A-8B (SEQ ID NO:1).

# EXHIBIT C

Serial No.: 09/884,098
Filed: June 20, 2001
Attorney Docket No.: 9426-022

## PENDING CLAIMS AS OF MARCH 7, 2003

1. A therapeutic agent comprising a bone sialoprotein ("BSP") promoter, a delivery vector and a toxic, therapeutic and/or heterologous coding sequence.

2. The therapeutic agent of claim 1, further comprising a prodrug.

3. The therapeutic agent of claim 2, wherein said prodrug is selected from the group consisting of acyclovir ("ACV") and gancyclovir ("GCV").

4. The therapeutic agent of claim 1, further comprising a glucocorticoid or an L-ascorbic acid.

5. The therapeutic agent of claim 1, wherein said BSP promoter comprises nucleotides -1349 to +69 depicted in Figures 8A-8B (SEQ ID NO:1).

6. The therapeutic agent of claim 1, wherein said delivery vector comprises a viral vector.

7. The therapeutic agent of claim 6, wherein said viral vector is an adenovirus.

8. The therapeutic agent of claim 1, wherein said delivery vector comprises a liposome.

9. The therapeutic agent of claim 1, wherein said toxic coding sequence is selected from the group consisting of thymidine kinase and cytosine deaminase.

10. The therapeutic agent of claim 1, wherein said therapeutic coding sequence is selected from the group consisting of growth factors, cytokines, therapeutic proteins, hormones and peptide fragments of hormones, inhibitors of cytokines, peptide growth and differentiation factors, interleukins, chemokines, interferons, colony stimulating factors and angiogenic factors.

11. The therapeutic agent of claim 1, wherein said heterologous coding sequence is a reporter gene.

12. The therapeutic agent of claim 11, wherein said reporter gene is a luciferase.

13. A method for identifying a test compound capable of modulating osteotropic-specific gene expression comprising:

(a) measuring the level of expression of a reporter gene under the control of a BSP regulatory region, or a transcriptionally active fragment thereof, in the presence and absence of said test compound,

such that if the level obtained in the presence of the test compound differs from that obtained in its absence, then a compound which modulates osteotropic-specific gene expression is identified.

14. The method of claim 13 wherein the reporter gene is luciferase.

15. A pharmaceutical composition comprising the test compound identified by the method in claim 13.

16. A method for delivery of a toxic and/or therapeutic molecule comprising, introducing into osteotropic cells of a subject a vector comprising a BSP regulatory region sequence, or transcriptionally active fragment thereof, operatively linked to a heterologous nucleic acid which encodes said toxic and/or therapeutic molecule.

17. A method for treating and/or ameliorating an osteotropic-related disease or disorder comprising introducing into osteotropic cells of a subject a vector comprising a BSP regulatory region sequence, or transcriptionally active fragment thereof, operatively linked to a heterologous nucleic acid whose gene product is capable of treating and/or ameliorating said disease or disorder.

18. A method for treating and/or ameliorating an osteotropic-related cancer or other proliferative disorder comprising introducing into a cell of said cancer or other proliferative disorder of a subject a vector comprising a BSP regulatory region sequence, or transcriptionally active fragment thereof, a delivery vector and a toxic, therapeutic and/or heterologous coding sequence whose gene product is capable of killing said cell.

19. The method of claim 18 wherein said cancer or other proliferative disorder is selected from the group consisting of osteosarcoma, prostate, breast, colon, lung, brain, multiple myeloma, thyroid, melanoma or any other disease or disorder with calcification potential.

20. The method of claim 18 further comprising introducing a prodrug.

21. The method of claim 20 wherein said prodrug is selected from the group consisting of ACV and GCV.

22. The method of claim 20 wherein said introducing comprises administration via direct application, or systemic application via intravenous administration, intra-arterial administration, intra-tumoral administration, perfusion and oral administration.

23. The method of claim 18, wherein said BSP regulatory region sequence comprises nucleotides -1349 to +69 depicted in Figures 8A-8B (SEQ ID NO:1).

24. The method of claim 23, wherein said BSP regulatory region sequence comprises a nucleotide sequence which hybridizes under highly stringent conditions to the complement of nucleotides -1349 to +69 depicted in Figures 8A-8B (SEQ ID NO:1).

25. The method of claim 23, wherein said BSP regulatory region sequence comprises a nucleotide sequence which hybridizes under moderately stringent conditions to the complement of nucleotides -1349 to +69 depicted in Figures 8A-8B (SEQ ID NO:1).

26. A method for preventing or delaying an osteotropic-related disorder comprising introducing into osteotropic cells of a subject a vector comprising a BSP regulatory region sequence, or transcriptionally active fragment thereof, operatively linked to a heterologous nucleic acid which encodes a therapeutic molecule which is capable of preventing or delaying said disorder.

27. A method for promoting bone repair comprising administering a polynucleotide to an area where bone repair is necessary, wherein said polynucleotide comprises a BSP regulatory region sequence, or transcriptionally active fragment thereof, a delivery vector and therapeutic coding sequence whose gene product is capable of promoting said bone repair.

28. The method of claim 27, wherein said therapeutic coding sequence is selected from the group consisting of growth factors, cytokines, therapeutic proteins, hormones and peptide fragments of hormones, inhibitors of cytokines, peptide growth and differentiation factors, interleukins, chemokines, interferons, colony stimulating factors and angiogenic factors.

29. A method for modulating immune functions comprising administering a polynucleotide to an area where modulation of immune function is necessary, wherein said polynucleotide comprises a BSP regulatory region sequence, or transcriptionally active fragment thereof, a delivery vector and therapeutic coding sequence whose gene product is capable of modulating immune functions.

30. The method of claim 29, wherein said therapeutic coding sequence is selected from the group consisting of interferons alpha, beta or gamma; tumour necrosis factor; granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (N-CSF), chemokines such as neutrophil activating protein NAP, macrophage chemoattractant and activating factor MCAF, RANTES, macrophage inflammatory peptides MIP-1a and MIP-1b, complement components and their receptors, accessory molecules such as 87.1, 87.2, ICAM-1.2 or 3 or cytokine receptors.